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THE REPRODUCTIVE BIOLOGY OF *MAGNOLIA GRANDIFLORA*

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ABSTRACT. The reproductive biology of *Magnolia grandiflora* was investigated at three localities in south Louisiana. Over the 3–4 day flowering period, the flowers of *M. grandiflora* exhibited changes in sex expression (protogyny), stigmatic receptivity (self- and cross-compatibility to self-incompatibility), UV reflectance (strong reflectance of the stigmas to strong reflectance of the androphore), and pollinator reward (a hexose-dominated stigmatic nectar to pollen). Although beetles were occasional floral visitors and carried pollen, bees (non-native *Apis mellifera* and indigenous *Lasioglossum bruneri*) were frequent floral visitors and were the only floral visitors whose behavior showed any correlation with the array of floral changes that occurred over the 3–4 day flowering period.

Key Words: *Magnolia grandiflora*, reproductive biology, halictid bees, bees

Magnolia is considered to be primitive among angiosperms. A widely accepted view of the evolution of the flower is the Euanthial or Anthostrobilus theory. This hypothesis asserts that the primitive flower is large, actinomorphic, solitary, white (sometimes pink or yellow), and borne terminally. The primitive flower may have a showy, undifferentiated perianth and numerous floral parts arranged spirally on an elongated axis. The stamens are broad, three veined, and each has four elongate microsporangia on its adaxial surface. The gynoecium is apocarpous, with conduplicate carpels that enclose a few ovules (Arber and Parkin 1907; Bessey 1897, 1915; Canright 1952, 1960; Maneval 1914). This widely held view of the magnolian flower has been considered a theoretical starting point for understanding angiosperm evolution (e.g., Cronquist 1981). However, recent phylogenetic analyses (Crane 1985; Donoghue and Doyle 1989; Loconte and Stevenson 1990, 1991; Nixon et al. 1994), and fossil evidence of

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early angiosperms (Crane et al. 1994, 1995; Friis et al. 1994, 1995; Taylor and Hickey 1992) suggest that the morphological features of the archetypical angiosperm flower are unresolved.

There is evidence to suggest that the diversification of angiosperms is associated with the diversification of the Apidae (Michener and Grimaldi 1988a, b; Crepet 1984, 1996; Crepet et al. 1991). The first occurrence of angiosperms, however, significantly predates the first occurrence of bees in the fossil record (Labandeira and Sepkoski 1993). The importance of the Diptera as early angiosperm pollinators has recently attracted attention (Kearns 1992; Kearns and Inouye 1993; Ren 1998). The Diptera have their origin in the Late Triassic–Early Jurassic (Rohdendorf 1974), prior to the origin and the diversification of angiosperms. Recent studies have shown that the floral foraging behavior of some groups of flies is similar to bees, and that certain groups of flies are faithful pollinators (Kearns 1992; Kearns and Inouye 1993; Ren 1998). Beetles are well known from the Mesozoic and occurred contemporaneously with the earliest known angiosperms. The large, unspecialized flowers of *Magnolia* are believed to be associated with the unspecialized "mess and soil" pollination syndrome of beetles (Faegri and van der Pijl 1979). Other investigators view beetle pollination in the Magnoliidae (Armstrong and Irvine 1990; Bernhardt and Thien 1987) and various other related groups (e.g. Cyclanthaceae; Beach 1982) as a specialized interaction involving floral modifications that are beneficial to the beetle pollinators. For example, in *Cyclanthus bipartitus* the bracts of the inflorescence produce a specialized high lipid tissue (50% by dry weight) that the beetles consume along with pollen (Beach 1982). Taxa that are unequivocally beetle pollinated frequently have floral modifications that specifically influence beetle behavior in the flower or inflorescence.

Despite a diversity of insect groups reported to visit *Magnolia,* most investigators have focused on the occurrence of beetles in the flowers of *Magnolia,* and on their role in pollen transfer (Baker and Hurd 1968; Delpino 1875; Dieringer and Espinosa 1994; Gibbs et al. 1977; Heiser 1962; Kikuzawa and Mizui 1990; Leppik 1975; Thien 1974). However, few of the floral features of *M. grandiflora* have been demonstrated to be specifically correlated with the floral behavior of the beetles.

The purposes of this study are as follows: a) to examine the floral characteristics that may function as insect attractants and/

or rewards, i.e., UV reflectance of floral parts, nectar secretion and composition, pollen availability, and the origin and longevity of the floral fragrance; b) to determine the self- and cross-compatibility of the flowers during anthesis and how this may relate to floral characteristics that are pollinator attractants and rewards; and c) to determine the kinds, numbers, and pollen loads of the various floral visitors, their behavior in the flowers of *Magnolia grandiflora,* and how this behavior may be related to floral characteristics that function as pollinator attractants and rewards.

MATERIALS AND METHODS

The investigation was carried out at three localities in south Louisiana. Site #1 consisted of 59 cultivated trees located throughout the city of Lafayette, Louisiana, a suburban habitat. Site #2 was a single tree in the Louisiana State Arboretum, located in Evangeline Parish, Louisiana. The 121.41 hectare arboretum is a secondary growth forest that is 75–80 years old. The tree at Site #2 was used for observing pollinators in the forest canopy. Access to the 30–40 m forest canopy was achieved using mountain climbing techniques (Perry 1978). Site #3 consisted of a monoculture of over 30 trees of varying ages at the Louisiana Nursery, Opelousas, Louisiana. The nursery is located in a rural area and the trees were grown under horticultural conditions.

Pollinator attractants and rewards. To determine UV reflectance, flowers were photographed with a 35 mm camera using a Kodak Wratten UV Filter No. 18A. This filter transmits long wave UV radiation (320–400 nanometers). High speed (ASA 400) T-Max 400 professional black and white film was used to record the reflectance patterns.

The origin of the floral fragrance was determined by dividing the flower into separate parts (gynoecium, androphore, upper petals, and lower petals) and placing the separated floral organs in sterilized glass jars for 20 minutes. Thirty volunteers rated each of the floral parts according to the intensity of the odor (0 being the least and 5 being the most fragrant).

The flowers used for hand pollinations (see below) were monitored for the presence of a stigmatic exudate (nectar) over the four-day flowering period. The nectar used in the sugar analysis was collected from first-day flowers from a variety of plants at

Site #1 by using capillary tubes. (Nectar production ceased after the first day of anthesis.) The nectar was immediately placed in a cooler with ice packs, transported to the lab, and refrigerated at 1°C. The nectar was run on 20 × 20 cm EM Reagent, Silica Gel 60 Type plates. The gel thickness was 0.25 mm. After the plates were spotted, the sugars were run over 15 cm with one or two ascents, against ten sugar standards of various dilutions of a 15% (w/v) stock solution. The standards included glucose, mannose, fructose, galactose, xylose, ribose, rhamnose, sucrose, maltose, and mannitol. The solvent used was a 9:6:3:1 solution of n-butanol : acetic acid : chloroform : water. The plates were developed at 100–110°C for 10–20 minutes after being sprayed with a 1:3 (v/v) solution of sulfuric acid : methanol. The presence or absence of protein in the nectar was determined by the application of 2% ninhydrin in acetone (w/v) to nectars spotted on a Thin Layer Chromotography (TLC) plate and then baked at 100°C.

The mean number of pollen grains per flower was estimated by removing thirty stamens from three flowers collected from three different trees at Site #1. Each of the thirty stamens was agitated with 0.01% Tween 20 in a known volume of water. Pollen grains were counted on a hemacytometer grid. The mean number of pollen grains per stamen and flower was calculated. In addition, the number of ovules per flower was recorded to derive an estimate of the pollen-ovule ratio (P/O).

Determination of self- and cross-compatibility. At Site #1, flowers were hand pollinated to determine stigmatic receptivity and cross- and self-compatibility. The pollinations were divided among ten treatments, 14–39 plants per treatment. Four of the treatments consisted of cross-pollinations on days 1–4 of anthesis, four consisted of self-pollinations on days 1–4, one treatment was an unpollinated bagged control for days 1–4, and one treatment was an unbagged control for days 1–4, to determine the seed set under natural conditions.

Prior to anthesis, the gynoecia of the hand pollinated flowers were bagged with tubular nylon hosiery. After the hand pollinations, the flowers were tagged. Pollen was collected for various pollinations in paper envelopes. The pollen was stored at −10°C with a desiccator (Williams 1980). To insure that all pollinations from the stored pollen had a similar level of viability, the duration of pollen viability was tested. Flowers were collected from three

different trees during the first morning of anthesis, the petals were removed, and the floral column with the stamens was placed on a sheet of paper. Within 24 hr., the anthers dehisced, the floral column was removed, one aliquot of pollen was stored at room temperature (21°C), and one aliquot was stored at −10°C. Pollen viability was tested from each of the aliquots daily for five consecutive days following anthesis using the method of Alexander (1969).

The gynoecia were collected as they ripened and the number of stigmas, the number of carpels setting seed, and the number setting two seeds were recorded. The percentage of ovules fertilized was calculated by dividing the total seed set by the total number of ovules (2/carpel) and multiplying by 100. The percentage of ovules fertilized for the ten treatments was compared using ANOVA (analysis of variance). A general linear model for unbalanced designs was chosen and a two-factor general linear model ANOVA was run on Minitab (Cruze and Weldon 1989). The model was fitted with two main effects, crossed and selfed treatments. The four days over which pollinations were made were treated as an interactive factor with four levels. F-ratios were compared at the 0.01 level of significance to detect differences among treatments. Significant differences between treatments were then tested using Tukey's multiple-comparison (W) procedure with Alpha = 0.05 (Cruze and Weldon 1989; Ott 1988).

Observations of insects. The types of insects and the number of visits by each taxon at Sites #1, #2, and #3 were recorded by direct observation or by videotape. Each of the three sites was monitored 14 times at equally spaced intervals between May 19 and July 29. Insect visitor data were recorded for an average of three hours per observation time, yielding a total of 42 hours of observations per site. Unfamiliar insects were collected for identification and five individuals of each species were collected as voucher specimens. All insects except thysanopterans were placed in a kill jar containing ethyl acetate. Thysanopterans were fixed in alcohol-glycerine-acetic acid (AGA) killing solution. After two weeks in the AGA, the thysanopterans were dehydrated in an alcohol series and mounted on microscope slides for identification (Borror et al. 1989). For each site the number of visits by each type of insect was recorded. The duration of the visits was recorded by direct observation and from time lapse and real-time

videotaping of the flowers. The pollen load per individual of each insect species was calculated as the mean number of grains carried on the insect specimens collected. The pollen carried on the corbicula of *Apis mellifera* was not available for stigmatic deposition and so was not included in the pollen load of this species. However, the pollen carried on the legs of halictid bees dislodged easily and was available for stigmatic deposition; thus these pollen grains were included in the pollen load for this species.

The relative importance (RI) of various insect species as pollinators was calculated using the following equation:

$$RI_i = \frac{P_i \times V_i}{(P \times V)} \times 100$$

where

RI_i = Index of relative importance of pollinator i,

P_i = Mean pollen load of pollinator i,

P = Sum of mean pollen loads of all insects,

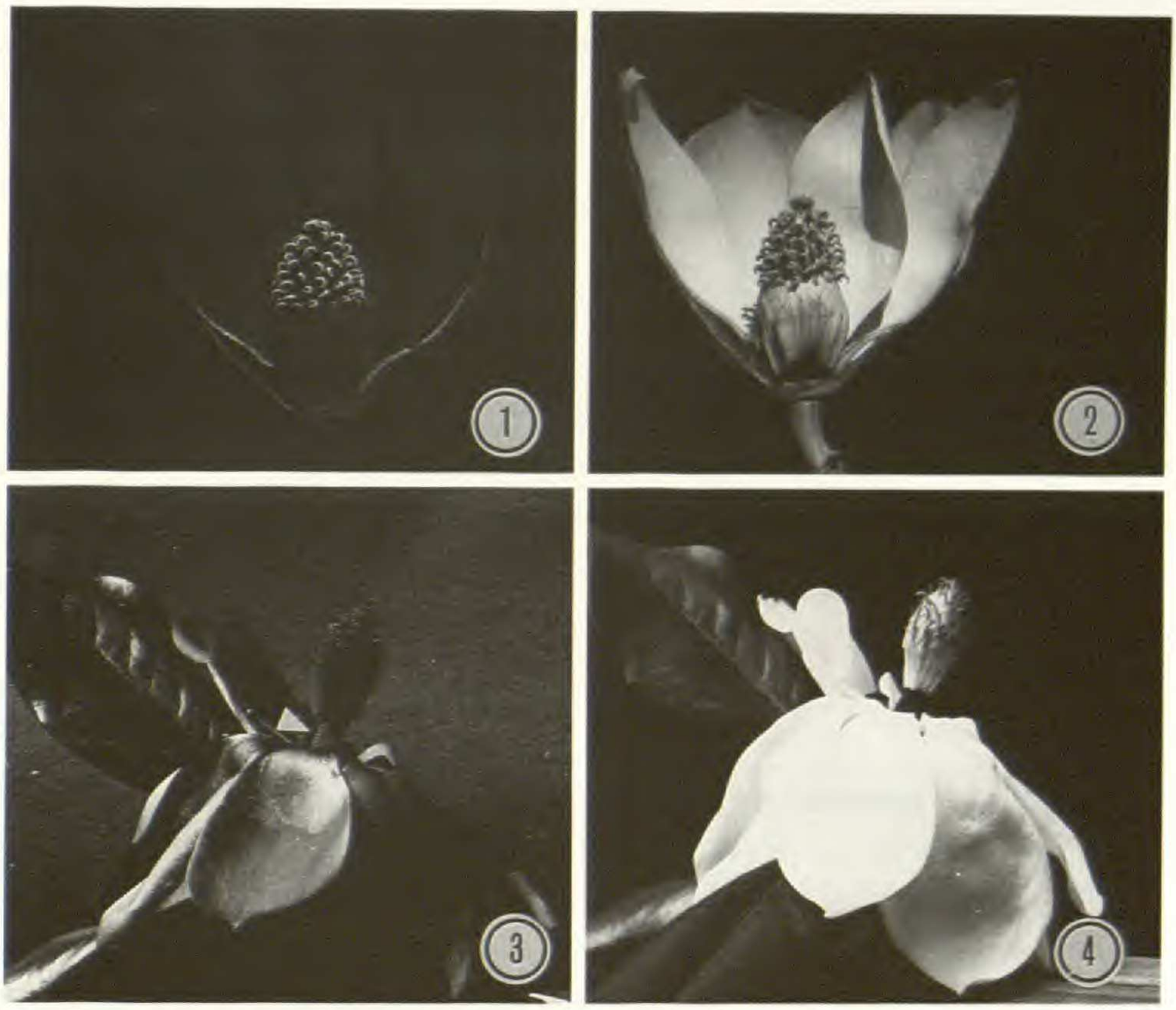
V_i = Number of visits of pollinator i,

V = Total number of insect visits.

Overall or total RI was calculated for each taxon by using the mean pollen load of the taxon over all three sites and the mean number of visits by that taxon at all three sites. A correlation coefficient was run on Minitab for all comparisons of insects recorded at the three sites.

RESULTS

Pollinator attractants and rewards. On the first day of anthesis the stigmas strongly reflected UV light (Figure 1, 2). On the second day of anthesis the stigmatic reflectance was evident, but had significantly faded in comparison to the first day of anthesis (Figure 3). By the third day of anthesis the UV reflectance of the stigmas was no longer evident. As the stigmatic reflectance faded on the second day of anthesis, the androphore, from which the stamens had abscised, reflected UV light in a checkered pattern (Figure 3, 4). The reflectance of the androphore was evident on the third day of anthesis, but was not detectable on the fourth



Figures 1–4. The two stages in *Magnolia grandiflora* floral phenology. 1. Ultraviolet photograph of a first day flower showing the reflective stigmatic crests. 2. Photograph of a first day flower taken without the UV filter. 3. Ultraviolet photograph of a second day flower showing the faded stigmas and "checkered pattern" of the reflective androphore (arrowhead). 4. Photograph of a second day flower without the UV filter.

day of anthesis. Throughout the four days of anthesis the corolla strongly absorbed UV light.

In the determination of the origin of the floral fragrance, 29 of the 30 volunteers rated the petals most fragrant, and the androphore least fragrant.

Nectar secretion associated with the stigmas occurred only on the first day of anthesis. Nectar was not evident on days 2–4. Using thin layer chromotography (TLC), three sugars were identified in the stigmatic exudate collected soon after the flowers opened on the first day of anthesis; these were glucose, fructose, and sucrose. Glucose and fructose, when visualized on the TLC plates, showed a similar intensity to the 15% (w/v) standard sugar solutions. Sucrose, although easily detectable, consistently exhib-

ited less intensity, suggesting that lesser amounts of sucrose were present in the stigmatic nectar than glucose and fructose. Nectar collected 3–6 hr. after anthesis had no detectable sucrose, indicating that as the nectar ages, the sucrose breaks down into hexose sugars. Nectars are known to contain protein (Baker and Baker 1975, 1983) and the nectar of *Magnolia grandiflora* tested positive for protein.

On the second day of anthesis the stamens dehisced and subsequently abscised from the androphore, falling into the cup-shaped petals. It was in the cup-shaped petals that magnolia pollen was available in abundance. The estimated mean number of pollen grains per flower was 58,000,000. The pollen-ovule ratio was estimated to range from 207,000:1 to 520,000:1 with an average of 412,000:1.

Self- and cross-compatibility. To insure that differences in seed set were not due to differences in pollen viability from day to day, pollen was tested for viability. On the day of anther dehiscence (day 2 of anthesis) a mean of 94.5% of the pollen grains were viable. There was no significant decrease in pollen viability for the five consecutive days following anthesis when pollen was stored at 21°C or −10°C.

Results of the hand pollinations showed that on the first day of anthesis the stigmas were receptive to geitonogamous and xenogamous pollinations. On the first day of anthesis the average seed set for xenogamous pollinations was 55%. The average seed set for geitonogamous pollinations was 38%, which was significantly lower than the cross-pollinated flowers (Table 1). On the second day of anthesis cross-pollinated flowers averaged 27% seed set, on the third day 13% seed set, and on the fourth day 2% seed set, which was not significantly different from the bagged, unpollinated control (Table 1). This indicates that receptivity to xenogamous pollinations was gradually reduced over the 3–4 day flowering period. In flowers that were self-pollinated, there was a significant decrease in percent seed set during the second day of anthesis, i.e., from 38% on the first day, to 5% on the second and third day of anthesis, and 3% on the fourth day of anthesis (Table 1). Seed set on days 2, 3, and 4 in the self-pollinated flowers was not significantly different from the unpollinated, bagged control. The unbagged, untreated control aver-

Table 1. Mean seed set resulting from hand pollinations of *Magnolia grandiflora* flowers. Cells with different bold letters **a**, **b**, **c**, **d** are statistically different at Alpha = 0.05. n = number of flowers/treatment; $\bar{x}$ = mean % seed set for each treatment; sd = standard deviation.

Treatments	Day 1	Day 2	Day 3	Day 4
Cross	n = 14 $\bar{x}$ = 55 **a** sd = 24	n = 16 $\bar{x}$ = 27 **b** sd = 20	n = 18 $\bar{x}$ = 13 **c** sd = 17	n = 14 $\bar{x}$ = 2 **d** sd = 3
Self	n = 20 $\bar{x}$ = 38 **b** sd = 24	n = 20 $\bar{x}$ = 5 **d** sd = 11	n = 12 $\bar{x}$ = 5 **d** sd = 15	n = 15 $\bar{x}$ = 3 **d** sd = 4
Control (bagged, unpollinated)	n = 39 $\bar{x}$ = 5 **d** sd = 11			
Control (unbagged, untreated)	n = 20 $\bar{x}$ = 34 **c** sd = 26			

aged 34% seed set, which is assumed to approximate seed set under natural conditions.

The results of the hand pollinations in the cross-pollinated flowers showed that from day 1 to day 4 there was a stepwise reduction in receptivity. Each day was significantly less receptive than the previous day, culminating on day 4, which was not significantly different from the unpollinated, bagged control. The flowers were self-compatible on the first day of anthesis and seed set in the self-pollinated flowers was similar to the percent seed set in the unbagged, untreated control. However, on the second, third, and fourth days of anthesis, self-pollinated seed set was not significantly different from the unpollinated, bagged control, indicating that the flower was essentially self-incompatible after the first day of anthesis.

Observations of insects. Seven orders of insects accounted for more than 99% of the insect visits to *Magnolia* flowers at all three sites. The correlation coefficient for all comparisons of the insects recorded at the three sites was greater than 0.93, indicating that the types of insects at each site, and the relative proportions of the insects visiting *Magnolia* at each site were similar. Frequencies of visits by the various insects varied among the sites, with Site #2 having almost twice as many insect visitors as Sites #1 and #3 over a similar time interval.

Hymenoptera and Thysanoptera accounted for 87% of the insect visits and Coleoptera, Diptera, Hemiptera, Homoptera, and Plecoptera for the remaining 13% of total insect visits (Table 2). Despite the occurrence of seven orders of insects visiting *Magnolia* flowers, Hymenoptera carried 98.9% of the pollen (excluding the pollen found in the corbiculae). The other six orders accounted for only 1.0% of the pollen carried (Table 3). The relative importance (RI) of each of the insect groups as pollen carriers shows the Hymenoptera with an RI of 99.98 over all three localities (Table 4). If the non-native honeybee (*Apis mellifera*) and the recently introduced *Magnolia* beetle (*Odontopus calceatus*) are excluded from the calculation, native halictid bees have an RI of 94.77 over all three localities (Table 5).

The Hymenoptera were the most frequent visitors of *Magnolia grandiflora* and carried the most pollen (Table 2, 3). Two species of ants belonging to the Formicidae accounted for 35 visits. The most common of these two ant species carried no pollen. The

Table 2. Number of insects per family visiting *Magnolia grandiflora* at each site, and combined over all sites. The number of visits per taxon are for the total 42 hr. of observations at each site. [1]Combined visits over all sites as a % of the total number of insect visits for all taxa.

	Number of Insect Visits				
Insect Taxon	Site #1	Site #2	Site #3	Combined	% of Total[1]
HYMENOPTERA					
Apidae	433	1,049	560	2,042	70.7
Halictidae	19	38	1	58	2.0
Other	36	8	0	44	1.5
TOTAL	488	1,095	561	2,144	74.2
COLEOPTERA					
Cantharidae	2	29	7	38	1.3
Curculionidae	90	0	62	152	5.3
Dermestidae	0	29	0	29	1.0
Mordellidae	41	32	3	76	2.6
Other	1	7	10	18	0.6
TOTAL	134	97	82	313	10.8
THYSANOPTERA					
Thripidae	166	198	3	367	12.9
ALL OTHER ORDERS	27	25	12	64	2.2

other species had an average pollen load of 2.75 grains each. At Site #1 five cuckoo bees (Apidae, Tribe Epeolini; absent from Sites #2 and #3) were collected, but no pollen was present. Two carpenter bees (*Xylocopa virginica*) visited flowers in the forest canopy at Site #2 and a single carpenter bee was observed at Site #1, and had a pollen load of 1350 pollen grains. The honey bee (*Apis mellifera*) dominated the floral fauna with an estimated 2042 visits out of 2888 recorded insect visits over the course of the field study. As many as ten honey bees were in a single flower at a time. Honey bees were most active in late morning to early afternoon. Honeybees averaged about 34 visits/hour as determined from videotaped flowers at Sites #2 and #3. The mean pollen load of *A. mellifera* (excluding the corbiculae) was estimated to be 22,200 pollen grains each (n = 4). The corbiculae were estimated to hold 73,000 pollen grains each (n = 4). A single species of solitary bee of the Andrenidae was collected six times at Site #2 and was found to carry a mean pollen load of 8300 pollen grains (n = 3). Andrenid bees were absent from Sites

Table 3. Statistical description of *Magnolia* pollen grains carried per insect. Taxa with less than 0.01% of total are excluded. [1]Standard error. [2]Percent of the total mean pollen load of all taxa combined. [3]Excluding pollen contained in the corbiculae. *No standard error due to sample size.

Insect Taxon	n	Mean	SE[1]	% of Total[2]
HYMENOPTERA				
Andrena vicina	3	8,333	1,816.30	17.10
Apis mellifera[3]	4	22,200	7,598	45.60
Lasioglossum bruneri	3	16,250	2,602.10	33.40
Unidentified Formicidae	4	3	1.60	0.01
Xylocopa	1	1,351	*	2.78
COLEOPTERA				
Cantharidae	5	97	39.30	0.20
Chrysomelidae	3	265	228.60	0.60
Curculionidae	5	26	10	0.05
Dermestidae	5	8	2.40	0.02
Mordellidae	5	23	7.60	0.05
Unidentified	3	7	7.30	0.02
DIPTERA				
Unidentified	10	2	1.20	0.01
HEMIPTERA				
Pyrrhocoridae	1	64	*	0.10
HOMOPTERA				
Cicadellidae	3	8	1.80	0.02

#1 and #3. The solitary halictid bee, *Lasioglossum bruneri,* was present at all three sites, with 19 individuals collected at Site #1, 38 at Site #2, and 1 at Site #3. These bees carried a mean pollen load of 16,300 grains (n = 3; excluding the corbiculae). A corbicula was found to carry approximately 44,300 grains (n = 1).

Thysanopterans were present at all three sites. At Site #1, 20.4% and at Site #2, 13.9% of all insects collected were thrips. Initially thrips were not noticed at Site #3, however thrips were later collected from preserved flowers. The Thysanoptera were too numerous and small to count in the field and were collected from preserved flowers at the end of the field season. The number of thrips does not represent the abundance of these insects over the entire study period. The 400–500 μm thrips collected from day 1 flowers (female phase) had pollen grains adhering to their bodies, suggesting that thrips do transfer pollen from flower to

Table 4. Relative importance (RI) of pollinators by family (calculated using all insects). Families with an overall pollinator RI less than 0.01 are excluded.

	Relative Importance of Pollinators			
Insect Taxon	Site #1	Site #2	Site #3	Overall
HYMENOPTERA				
Apidae	97.7	98.01	99.87	98.45
Andrenidae	0	0.11	0	0.06
Halictidae	2.28	1.87	0.09	1.47
COLEOPTERA				
Chrysomelidae	0.01	0.01	0.04	0.02

flower. Despite their high abundance, thrips carried an average of 0.3 pollen grains each.

The highest number and relative abundance of coleopterans was found at Site #1 on cultivated trees in the suburban habitat. One of the most common beetles was the recently introduced magnolia beetle, *Odontopus calceatus*. This species was also re-

Table 5. Relative importance (RI) of pollinators by family (calculated with *Apis mellifera* and *Odontopus calceatus* excluded). Families with an overall RI less than 0.01 are excluded.

	Relative Importance of Pollinators			
Insect Taxon	Site #1	Site #2	Site #3	Overall
HYMENOPTERA				
Andrenidae	0	5.36	0	3.55
Halictidae	98.98	93.66	69.74	94.77
Xylocopa	0.15	0.14	0	0.14
COLEOPTERA				
Cantharidae	0.01	0.06	0.39	0.05
Chrysomelidae	0.55	0.52	29.41	1.21
Dermestidae	0	0.06	0	0.04
Mordellidae	0.27	0.15	0.40	0.19
DIPTERA				
Diptera/Other	0	0.01	0.01	0.01
HEMIPTERA				
Pyrrhocoridae	0	0.03	0	0.02
THYSANOPTERA				
Thripidae	0.02	0.01	0.01	0.02

corded from Site #3, but was absent from the forest canopy (Site #2). Magnolia beetles carried a mean pollen load of 26 grains (n = 5). One species of Cantharidae was common at Site #2 and carried the largest pollen load (an average of 97 grains, with a maximum of 203 grains) of all the coleopterans collected. Members of the Mordellidae occurred at all sites and were common at Sites #1 and #2. A total of 41 individuals were collected at Site #1, and 32 individuals at Site #2. Members of the Dermestidae were collected at Site #2 and had an average pollen load of 8 grains. Beetles represented by a single individual were not identified. Coleopterans were observed to remain in flowers for long periods of time, and many remained in the flower for the entire time the flower was observed (up to 24 hr.). The random examination of over 400 flowers on the first day of anthesis (female phase) resulted in the observation of two beetles, one member of the Cantharidae and one of the Lampyridae.

Dipterans were present at all sites although no one species was most common. Dipterans were most abundant at Site #1 where 16 of 25 dipterans collected were *Plecia nearctica* (Bibionidae). Other dipterans collected included three species of the Asilidae and four unidentified taxa at Site #1. At Site #2 three species of the Sciomyzidae and three unidentified species were collected. In addition 16 other taxa were observed, but were not captured for identification. Dipterans at Site #3 included two members of the Phoridae, six species of the Bibionidae, and one unidentified species. Of the dipterans collected, most carried no pollen and three species carried five, seven, and eight grains, respectively.

Of the remaining insects collected, one plecopteran of the Perlidae was collected at Site #3, but carried no pollen. Four homopterans, all members of the Cicadellidae, were collected and carried an average of 8 pollen grains/insect. Two species of hemipterans were collected; *Nezara viridula* was collected at Sites #2 and #3 and carried no pollen, and *Euryophthalmus succinctus* was collected from Site #2 and was carrying 64 pollen grains.

DISCUSSION

The flowers of *Magnolia grandiflora* exhibited interactions among floral movements, pollinator attractants and rewards, and stigmatic receptivity. On the first day of anthesis the flowers opened early in the morning presenting the receptive stigmas (fe-

male phase). Concomitant with anthesis was a conspicuous stigmatic nectar and a strong floral fragrance, which emanated from the petals. Three sugars were identified in the stigmatic exudate. Fresh nectar (nectar collected during the early morning) was comprised of glucose and fructose with a lesser amount of sucrose (i.e., a hexose-dominated nectar; Baker and Baker 1975). Baker and Baker (1975, 1983) have shown that hexose-dominated nectars also contain measurable amounts of protein. The stigmatic nectar of *M. grandiflora* tested positive for protein. Nectar that had been exposed to air temperature for 3–6 hr. was comprised of only glucose and fructose; the sucrose became undetectable using thin layer chromotography. This suggests that the sucrose may have been enzymatically broken down into hexose sugars as the nectar aged. Baker and Baker (1975, 1983) have demonstrated that hexose-dominated nectars are usually associated with the dish-bowl flower morphology, and pollination by short-tongued bees (e.g., honeybees and halictid bees) or flies. The reflectance of UV light was particularly strong by the stigmas (Figure 1, 2). The highly reflective stigmas occurred against a corolla that strongly absorbed UV light (Figure 1, 2). The floral fragrance and the highly reflective stigmas in combination with the hexose-dominated stigmatic nectar are floral attractants that can potentially direct pollinators to the receptive stigmas in first-day flowers. First-day stigmas were receptive to geitonogamous and xenogamous pollinations (Table 1). Based on our field observations and videotape of floral visitors at all three sites during the first day of anthesis, 88% of the honeybee (n = 366) and 96% of the halictid bee (n = 23) landings were on the stigmas. The bees spent an average of 1 minute 14 seconds (n = 44) in first-day flowers. We interpret this bee behavior as a response to olfactory (floral fragrance) and visual (UV reflectance of the stigmas) floral cues that are associated with nectar as the reward. After 1500 hours on the first day of anthesis the flowers began to close, were fully closed by 1900–2000 hours, and remained closed until the early morning of the second day of anthesis.

On the second day of anthesis the flowers re-opened early in the morning. At this time the anthers dehisced and then abscised from the androphore throughout the day, falling into the cup-shaped petals. The flowers remained open continuously for the next 2–3 days. The floral fragrance remained detectable. The UV reflectance of the stigmas had faded but was still detectable (com-

pare Figure 1 with Figure 3), however, the reddish-purple androphore strongly reflected UV light in a checkered pattern after all of the stamens had abscised (Figure 3, 4), i.e., the conspicuous UV reflectance had shifted from the stigmas to the androphore. The stigmatic nectar was no longer evident, however, pollen was now available in abundance. The estimate of the mean number of pollen grains per flower was approximately 58,000,000. It appears that the pollinator reward had also shifted from nectar to pollen (Yasukawa et al. 1992). The pollen/ovule ratio (P/O) was calculated for three flowers from three different trees. The P/O averaged 412,000:1. The high P/O is not consistent with the occurrence of constant pollinators and suggests pollen limitation (Cruden 1977; Cruden and Miller-Ward 1983). However, in *Magnolia* the pollen was not presented while the stamens were attached to the androphore, but on the cup-shaped petals after they had abscised. There was frequent loss of pollen-laden stamens due to disturbance of the flowers (e.g. by wind). The high P/O may compensate for the losses due to this unusual type of pollen presentation. Stigmatic receptivity remained high for cross-pollination, however, self-compatibility was greatly reduced (Table 1). There was no significant difference in seed set between the bagged unpollinated control and the self-pollinated flowers on the second day of anthesis. Seed set in cross-pollinated ovules was lower than cross-pollinated ovules on the first day, but was significantly higher than the self-pollinated ovules on the second day of anthesis (Table 1). The shift in pollinator attractants and reward on the second day suggests that a concomitant shift in pollinator behavior in the flower should also occur. Based on our observation and videotape of pollinators at all three sites on the second day of anthesis, 62% of the honeybee (n = 282) and 71% of the halictid bee (n = 7) landings were on the reddish-purple androphore, the bees then made a 180° turn and began to forage for pollen on the abscised stamens held in the cup-shaped petals. Once in the cup-shaped petals, the bees often held the stamens like lollipops, using their forelimbs to scrape pollen from the anthers. Honeybees spent an average of 2 minutes 52 seconds (n = 50) in second-day flowers. Thus, the shift in the visual cue for pollinators (UV reflectance of the stigmas to reflectance of the androphore) and the shift in the pollinator reward (from nectar to pollen) correlated with changes in the foraging behavior of bees in the flowers from the first to the second day of anthesis.

On the third day of anthesis floral fragrance was still evident, but had faded dramatically and the stigmas no longer reflected UV light. The flower was still cross-compatible (Table 1), however receptivity was reduced further, though it was still significantly higher than the bagged, unpollinated control. The behavior of the hymenopteran floral visitors was similar to the behavior observed in second-day flowers.

On the fourth day of anthesis floral fragrance and UV reflectance were no longer evident and the flower was no longer receptive. In addition, a majority of the stamens (and hence the pollen) had been lost from the wilting, cup-shaped petals. The number of hymenopteran visits also dropped off dramatically.

In our observations at all three sites (including videotape taken at Sites #2 and #3), hymenopterans were the only floral visitors whose behavior showed any correlation with the array of floral changes that occurred over the 4-day period in the flowers of *Magnolia grandiflora.* It is expected from our investigation of floral movements, stigmatic receptivity, and the pollinator attractants and rewards, that hymenopterans play an important role in the pollination of *Magnolia.*

Based on our observation of floral visitors at all three sites, the most frequent visitors of *Magnolia grandiflora* were hymenopterans (bees; Table 2). Coleopterans are considered the primary pollinators of various magnoliid genera and species of *Magnolia* (Baker and Hurd 1968; Delpino 1875; Dieringer and Espinosa 1994; Gibbs et al. 1977; Heiser 1962; Kikuzawa and Mizui 1990; Leppik 1975; Thien 1974). In this study beetles accounted for 10.8% of all insect visits. Beetles consistently carried far less pollen than the bees, and frequently carried pollen of other species (Table 3). The hymenopterans in this study comprised 74.2% of the total insect fauna observed on *Magnolia* flowers. *Apis mellifera* outnumbered other insect visitors and accounted for 70.7% of all insect visits. Honey bees are not indigenous to North America; however, they were attracted to the flowers of *M. grandiflora* and were capable of pollinating the flowers. This suggests that their behavior is similar to the native pollinators. At all three sites, native *Lasioglossum bruneri* were second only to honeybees in relative importance and thus may be a common natural pollinator of *M. grandiflora* (Table 4, 5).

Beetles were floral visitors and carried pollen. They may have transferred pollen by a "mess and soil" pollination syndrome.

Bees, however, were frequent floral visitors and carried large amounts of pollen. Bees were the only pollinators that exhibited behavior in the flowers that was associated with the changes in sex expression, stigmatic receptivity, and changes in pollinator attractants and rewards over the flowering period. The changes in sex expression, stigmatic receptivity, shifts in UV reflectance of floral parts, and pollinator rewards, coupled with the influence these changes had on the behavior of the hymenopteran visitors in the flowers, suggests that despite its size and its presumed archaic flower morphology, *Magnolia* has evolved specialized floral features that effectively influence the foraging behavior of more specialized pollinators (i.e., bees).

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LITERATURE CITED

ALEXANDER, M. P. 1969. Differential staining of aborted and non-aborted pollen. Stain Technol. 44: 117–122.

ARBER, E. A. N. AND J. PARKIN. 1907. On the origin of angiosperms. J. Linn. Soc., Bot. 38: 29–80.

ARMSTRONG, J. E. AND A. K. IRVINE. 1990. Functions of staminodes in the beetle-pollinated flowers of *Eupomatia laurina*. Biotropica 22: 429–431.

BAKER, H. G. AND I. BAKER. 1975. Studies of nectar-constitution and pollinator-plant coevolution, pp. 100–140. *In:* L. E. Gilbert and P. H. Raven, eds., Coevolution of Animals and Plants. Univ. Texas Press, Austin, TX.

——— AND ———. 1983. Floral nectar sugar constituents in relation to pollinator type, pp. 117–141. *In:* C. E. Jones and R. J. Little, eds., Handbook of Experimental Pollination Biology. Van Nostrand-Reinhold, Princeton, NJ.

——— AND P. D. HURD. 1968. Intrafloral ecology. Ann. Rev. Entomol. 13: 385–414.

BEACH, J. H. 1982. Beetle pollination of *Cyclanthus bipartitus* (Cyclanthaceae). Amer. J. Bot. 69: 1074–1081.

BERNHARDT, P. AND L. B. THIEN. 1987. Self-isolation and insect pollination in the primitive angiosperms: New evaluations of older hypotheses. Pl. Syst. Evol. 156: 159–176.

BESSEY, C. E. 1897. Phylogeny and taxonomy of the angiosperms. Bot. Gaz. 24: 145–178.

———. 1915. The phylogenetic taxonomy of flowering plants. Ann. Missouri Bot. Gard. 2: 109–164.

BORROR, D. J., C. A. TRIPLEHORN, AND N. F. JOHNSON. 1989. An Introduction to the Study of Insects. Saunders College Publ., Philadelphia, PA.

CANRIGHT, J. E. 1952. The comparative morphology and relations of the Magnoliaceae. I. Trends of specialization in the stamens. Amer. J. Bot. 39: 484–497.

———. 1960. The comparative morphology and relations of the Magnoliaceae. III. Carpels. Amer. J. Bot. 47: 145–155.

CRANE, P. R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. Ann. Missouri Bot. Gard. 72: 716–793.

———, E. M. FRIIS, AND K. R. PEDERSEN. 1994. Palaeobotanical evidence on the early radiation of magnoliid angiosperms. Pl. Syst. Evol., Suppl. 8: 51–72.

———, ———, AND ———. 1995. The origin and early diversification of angiosperms. Nature 374: 27–33.

CREPET, W. L. 1984. Advanced (constant) insect pollination mechanisms: Patterns of evolution and implications vis-a-vis angiosperm diversity. Ann. Missouri Bot. Gard. 71: 607–630.

———. 1996. Timing in the evolution of derived floral characters: Upper Cretaceous (Turonian) taxa with tricolpate and tricolpate derived pollen. Rev. Palaeobot. Palynol. 90: 339–359.

———, E. M. FRIIS, AND K. C. NIXON. 1991. Fossil evidence for the evolution of biotic pollination. Philos. Trans., Ser. B 333: 187–195.

CRONQUIST, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia Univ. Press, NY.

CRUDEN, R. W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

——— AND S. MILLER-WARD. 1983. Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: An hypothesis. Evolution 35: 964–974.

CRUZE, E. AND J. WELDON. 1989. Minitab Reference Manual. Data Tech Industries, Inc., Valley Forge, PA.

DELPINO, F. 1868–1875. Ulteriori observazioni e considerazioni sulla sicogamia nel regno vegetale. I–II. Attc. Soc. Ital. Sci. Nat. 11–12.

DIERINGER, G. AND J. E. ESPINOSA S. 1994. Reproductive ecology of *Magnolia schiedeana* (Magnoliaceae), a threatened cloud forest tree species in Veracruz, Mexico. Bull. Torrey Bot. Club 121: 154–159.

DONOGHUE, M. J. AND J. A. DOYLE. 1989. Phylogenetic studies of seed plants and angiosperms based on morphological characters, pp. 181–193. *In:* B. Fernholm, K. Bremer, and H. Jornwall, eds., The Hierarchy of Life. Excerpta Medica, Amsterdam.

FAEGRI, K. AND L. VAN DER PIJL. 1979. The Principles of Pollination Ecology, 3rd ed., Pergamon Press, NY.

FRIIS, E. M., K. R. PEDERSEN, AND P. R. CRANE. 1994. Angiosperm floral structures from the Early Cretaceous of Portugal. Pl. Syst. Evol., Suppl. 8: 31–49.

———, ———, AND ———. 1995. *Appomattoxia ancistrophora* gen. et sp. nov., a new Early Cretaceous plant with similarities to *Circaester* and extant Magnoliidae. Amer. J. Bot. 82: 933–943.

GIBBS, P. E., J. SEMIR, AND N. D. DA CRUZ. 1977. Floral biology of *Talauma ovata* St. Hil. (Magnoliaceae). Ci. & Cult. 29: 1436–1441.

HEISER, C. B. 1962. Some observations on pollination and compatibility in *Magnolia.* Proc. Indiana Acad. Sci. 72: 259–266.

KEARNS, C. A. 1992. Anthophilous fly distribution across an elevation gradient. Amer. Midl. Naturalist 127: 172–182.

——— AND D. W. INOUYE. 1993. Pistil-packing flies. Nat. Hist. 102: 30–37.

KIKUZAWA, K. AND N. MIZUI. 1990. Flowering and fruiting phenology of *Magnolia hypoleuca.* Pl. Spec. Biol. 5: 255–261.

LABANDEIRA, C. C. AND J. J. SEPKOSKI. 1993. Insect diversity in the fossil record. Science 261: 310–315.

LEPPIK, E. E. 1975. Morphogenetic stagnation in the evolution of magnolia flowers. Phytomorphology 25: 451–464.

LOCONTE, H. AND D. W. STEVENSON. 1990. Cladistics of the Spermophyta. Brittonia 42: 197–211.

——— AND ———. 1991. Cladistics of Magnoliidae. Cladistics 7: 267–296.

MANEVAL, W. E. 1914. The development of *Magnolia* and *Liriodendron,* including a discussion of the primitiveness of the Magnoliaceae. Bot. Gaz. 57: 1–31.

MICHENER, C. D. AND D. A. GRIMALDI. 1988a. A *Trigona* from Late Cretaceous amber of New Jersey (Hymenoptera: Apidae: Meliponinae). Amer. Mus. Novit. 2917: 1–10.

——— AND ———. 1988b. The oldest fossil bee: Apoid history, evolutionary stasis, and the antiquity of social behavior. Proc. Natl. Acad. Sci. U.S.A. 85: 6424–6426.

NIXON, K. C., W. L. CREPET, D. A. STEVENSON, AND E. M. FRIIS. 1994. A reevaluation of seed plant phylogeny. Ann. Missouri Bot. Gard. 81: 484–533.

OTT, L. 1988. An Introduction to Statistical Methods and Data Analysis, 3rd ed., PWS-Kent Publishing Company, Boston, MA.

PERRY, D. R. 1978. A method of access into the crowns of emergent and canopy trees. Biotropica 10: 155–157.

REN, D. 1998. Flower-associated Brachycera flies as fossil evidence for Jurassic angiosperm origins. Science 280: 85–88.

ROHDENDORF, B. 1974. The Historical Development of Diptera. Univ. Alberta Press, Alberta, Canada.

TAYLOR, D. W. AND L. J. HICKEY. 1992. Phylogenetic evidence for the herbaceous origin of angiosperms. Pl. Syst. Evol. 180: 137–156.

THIEN, L. B. 1974. Floral biology of *Magnolia.* Amer. J. Bot. 61: 1037–1045.

WILLIAMS, P. H. 1980. Bee-sticks, an aid in pollinating Cruciferae. Hort. Sci. 15: 802–803.

YASUKAWA, S., H. KATO, R. YAMAOKA, H. TANAKA, H. ARAI, AND S. KAWANO. 1992. Reproductive and pollination biology of *Magnolia* and its allied genera Magnoliaceae. I. Floral volatiles of several *Magnolia* and *Michelia* species and their roles in attracting insects. Pl. Spec. Biol. 7: 121–140.